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Extraction of Inulin from Various Yam Tubers (*Dioscorea spp.*)

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Abstract

Research on the extraction and precipitation of inulin from four species of yam tubers (*Dioscorea spp.*) was performed. The purpose of this study was to find the optimum condition of inulin extraction from several species of *Dioscorea* spp. Four species of yam tubers (*Dioscorea* spp.) were used in this study i.e.: *Dioscorea esculenta*, *Dioscorea bulbivera*, *Dioscorea alata* and *Dioscorea opposita*. The locally grown yam tubers were obtained from East Java, Indonesia. Extraction of inulin was conducted using hot water followed by precipitation at various temperatures. Briefly, yam tuber was mixed with hot water (90 °C) at ratio 1:20, decanted in water bath for 1 hour and then filtered. The filtrate was precipitated at various temperatures (0 °C, -10 °C, -20°C and -30°C). After thawing, the filtrate was then centrifuged at 1500 rpm for 15 min, precipitated and dried at 60 °C for 6 hours.The dried inulin were analysed for its water content, solubility and water absorbtion. The purity of dried inulin was determined by HPLC with Aminex HPX-87c column. All of the data were analyzed using Analysis of Variance (ANOVA) and further tested by Duncan Multiple Range Test (DMRT). The results showed that *Dioscororea esculenta* tuber had the highest yield of extracted dried inulin (21.08%). Optimum condition for inulin extraction was obtained at precipitation temperature of -20 °C. The water content in dry inulin was 13.89% with 99.09% solubility and water of absobtion of 12.39%. The purity of the dried inulin was 92.77%.

Key words : inulin, extraction, precipitation, Dioscorea spp., Dioscorea esculenta.

Introduction

Inulin is a polymer of fructose units with terminal groups of glucose. The units in the inulin fructose linked by β bond (1,2) glycosidic linkages, which can not be digested by enzymes in the digestive system of mammals that reaches the colon without changing its structure (Roberfroid, 2005). Inulin and Fructo-oligosaccharides (FOS) can significantly increase the growth of bifidobacteria (Gibsonand others 1995; and Pompei and others 2008). Pompei and others (2008) showed that inulin enhanced the growth of *Bifidobacterium adolesentis, Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium longum, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus delbruechii* and inhibited the growth of *E. coli* and *Clostridia.*

Inulin can be commercially produced from chicory roots (*Cichorium intybus*), however chicory plant is not found in Indonesia. In addition, inulin has not been produced in Indonesia yet and for the industrial and research purpose it is mostly imported. Therefore, it is important to study the potential source of inulin from local raw materials. One of the many types of plants that grow in Indonesia and potentially contains high amount of inulin is *Dioscorea spp.* (Anonymous, 2009; www.wikipedia.org.)

Study showed that inulin powder can be produced principally by extracting, filtrating, refrigerating/freezing, centrifugation, precipitating and then drying the chicory roots. (Rogge, 2005; Bosscher and Ghent, 2005; Toneli and others 2008). Kim and others (2001) showed that at a temperature of 25 °C inulin almost insoluble in water, but its solubility increased significantly with increasing temperature. Berghofer and others (1993) showed that inulin solution (40% by weight) which was cooled from 95°C to 4°C for more than 30 hours will precipitate or crystallize as colorless/pale compound and can be separated by filtration. Hebette and others (1998) showed that a solution of concentrated (30-45% by weight) inulin cooled at 1 ° C/min or 0.25 ° C/min from 96 ° C to 20 ° C, could formed suspended semicristaline inulin in water.

The objective of the research was to determine the optimum condition of inulin extraction from several species of *Dioscorea spp.*

Materials and Method

Materials used in this study were 10 types of four species of *Dioscorea spp.* tubers namely *Dioscorea esculenta, Dioscorea bulbifera, Dioscorea alata* and *Dioscorea opposita.* The tubers were obtained from various regions in East Java, Indonesia. The materials for analysis included cysteine, H2SO4, karbazol (Sigma Chemical), ethanol, inulin standards (Sigma).

Analysis of inulin content (Kierstan, 1980; Widowati, 2005)

One ml of the inulin filtrate plus 0.2 ml 1.5% of cysteine and 6 ml 70% H2SO4 were mixed. The mixture were shaken, added with 0.2 ml 0.12% of karbazol solution, heated at 60°C for 10 minutes in a waterbath and cooled. The absorbance was then measured at a wavelength of 560 nm. Standard curve was made by measuring the absorbance of the samples containing inulin standard.

Extraction and precipitation of Inulin (Park, 2000; Toneli, 2008; Modification)

Dioscorea tuber was cleaned, washed, peeled and cut into small pieces, then blended with hot water at temperature of 90 °C, for 1 hour, in shaker waterbath. Concentrated solution was frozen at temperature of 0 °C, -10 °C, -20 °C and -30 °C. The solution was cooled and thawed at 8 °C for 42 hours. The concentrates were centrifuged at 1500 rpm, for 15 minutes until the white precipitates obtained and separated. White precipitates were then dried at 60 °C and mashed.

Inulin Purity Test (Toneli, 2008)

The inulin concentration at the precipitated phases was determined through HPLC analysis with Aminex columns HPX-87c (250mm x 4mm), a refractive index Waters model 410 detectors and a LCHE Waters model M-45 pump. Water was taken as the mobile phase at a rate of 0.3ml/min, with an injected volume of 20 liters. The temperature of the column was set at 80°C and at 40°C for the detector. The concentration quantification was based on inulin standards prepared with Fluka sample (Bio Chemika).

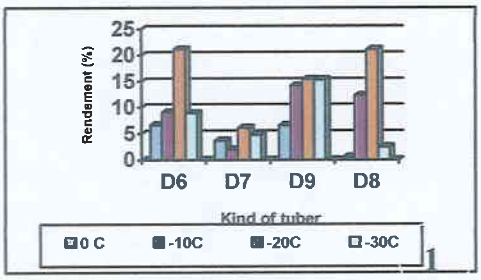
Results and Discussion

Inulin rendement

The highest inulin yield (21.08%) was obtained from *Dioscorea esculenta* (*gembili*) at precipitation temperature of -20 °C and the lowest (6.10%) was obtained from *Dioscorea alata* (yellow yam) with the same precipitation temperature. Average values of inulin rendement were presented in Figure 1. The amount of inulin obtained was different for each variety and the temperature of precipitation. This may be caused by different inulin molecular weight (MW) from each tuber. The solutes molecular weight (MW) will affect the speed of deposition of these substances and the freezing point of solution. Inayati (2007) showed that the amount of settled solute particles correlated with the high molecular weight (MW) owned by such solutes.

Water Content

The highest water content (13.19%) of inulin was obtained from *Dioscorea esculenta* (gembili) at the precipitation temperature of -10 °C and the lowest water content (5.89%) was obtained at the precipitation temperature of -20 °C. The average value of water content in dried inulin is presented in Figure 2. The difference of water content might be due to differences in the number of hydroxyl (OH) in each inulin powder. The more hydroxyl group is possessed by inulin, the more water is bound, because the hydroxyl group (OH) is polar (easy to absorb water).



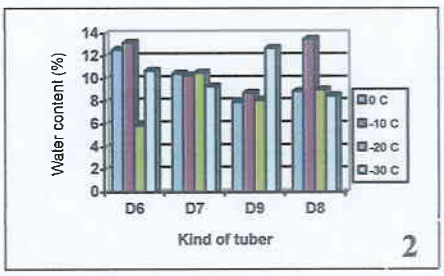


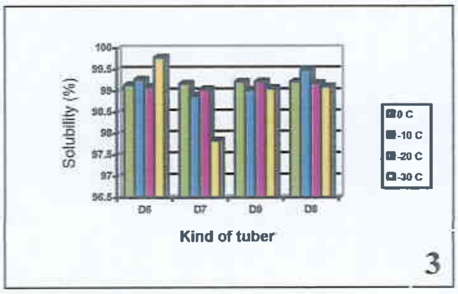
Figure 1. Inulin Rendement; 2. Water Content

Solubility of Dried Inulin

Different types of tuber and precipitation temperatures produced dried inulin that had a relatively high solubility of 97.84% (*Dioscorea alata*) to 99.78% (*Dioscorea esculenta).* There were no significant differences between each treatment. The average value of dried inulin solubility is presented in Figure 3. Inulin solubility is affected by its hydroxyl group (OH) cluster owned by inulin and its polarity.

Water Absorption

All types of tubers at different precipitation temperatures produce dried inulin that had low water absorption of 4.1% (*Dioscorea opposite*/white tuber) to 16.30% (*Dioscorea bulbifera*/gembolo). The average values of water absorption of dry inulin are presented in Figure 4. Water absorption of inulin is caused by hydroxyl group of inulin. Because the hydroxyl group is polar, it is easy to absorb water (hygroscopic).



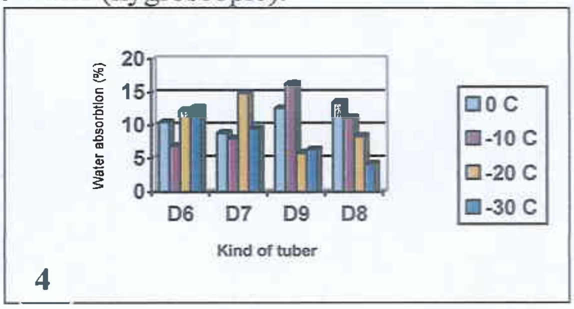
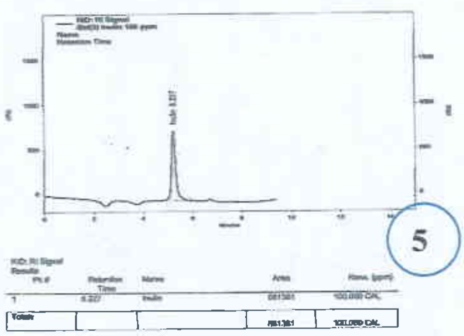


Figure 3. Solubility of Inulin; 4. Water Absorbtion of Inulin

Purity of Inulin

Dried inulin from *Dioscorea esculenta* tuber has purity of 92.99%, compared to standard inulin which was detected at retention time of 5.212 minutes. Chromatograms of standard inulin and dry inulin are presented in Figure 5 and 6.



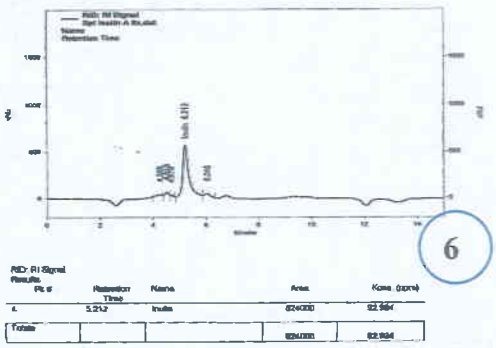


Figure 5. Inulin standard curve; 6. Dry Inulin

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